component of the cytoplasmic dynein biological motor complex has a relatively large number of interacting partners and fits the description of a hub. Partner proteins of LC8 include the intermediate chain (IC) subunit of cytoplasmic dynein, Chica, a mitotic spindle-associated protein, Nup159, a component of the yeast nuclear pore complex, and a wide array of proteins with roles in apoptosis, enzyme regulation and viral pathogenesis. Linear motifs mediate binding between LC8 and its partner proteins, but while partner proteins such as dynein IC have only one recognition motif, others such as Chica and Nup159 are enriched with multiple interacting motifs.

To better understand the importance of multiple recognition motifs in the LC8 interaction network, we have initiated structural and thermodynamic studies of the LC8/Chica and LC8/Nup159 complexes. Chica and Nup159 have four and six putative recognition motifs respectively, nestled within segments that are intrinsically disordered. Our results indicate that LC8 forms a dynamic complex with both proteins, binding only three (Chica) or five (Nup159) of the putative recognition motifs. Furthermore, we show that only three LC8 dimers are needed for optimal stability of the Nup159/LC8 complex, and suggest that the evolutionary adaptation of multiple LC8 recognition motifs imparts to the complex other properties such as rigidity. These findings extend the repertoire of functions of intrinsically disordered proteins to fine-tuning and versatile assembly of higher order macromolecular complexes.

3475-Pos Board B203

Conformational Allostery in Nuclear Receptor/Coregulator Transcriptional Complexes

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By recruiting chromatin remodeling machinery to promoter regions of target genes, nuclear receptors (NRs) act as ligand-dependent transcriptional protein scaffolds, exerting powerful influences on all aspects of physiology. The critical mediators that bridge NRs to the transcriptional machinery are called NR coregulators. NR coregulators are broadly classified into coactivators or corepressors depending on their ability to promote or repress gene transcription. Coactivators contain a cluster of "LXXLL" motifs in a flexible/disordered region called the Receptor Interaction Domain (RID) that mediates their interactions with NRs. Because there is very little atomic level structural information on disordered coactivator RIDs, the physical mechanism driving the interaction between coregulator RIDs and NR transcriptional complexes is poorly understood. We are studying the interaction of Steroid Receptor Coactivator-2 (SRC-2) with the PPARγ-RXR transcriptional complex as a model system for understanding NR/coregulator interactions on the atomic level. SRC-2 is a critical coactivator in the PPARγ-driven differentiation of mesenchymal stem cells into fat cells, a pathway that is a target for therapeutic intervention of metabolic syndrome and type 2 diabetes. Using biochemical, biophysical and solution NMR approaches, we have begun to probe the interaction of the SRC-2 RID with the full-length PPARγ-RXR complex bound to DNA (~150 kDa complex). Our studies reveal that the binding of DNA to PPARγ-RXR imparts a long-range conformational change that impacts the binding and conformation of SRC-2 RID. Importantly, our work extends the recent proposition that DNA can act as an allosteric effector of NR activity, indicating that DNA binding can affect the conformation of a coregulator bound to a NR transcriptional complex.

3476-Pos Board B204

Are Spider Silk Proteins a New Class of Intrinsically Disordered Proteins?

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Spider dragline silk is an outstanding biopolymer with a strength that exceeds steel by weight and a toughness greater than high-performance fibers like Kevlar. For this reason, understanding how a spider converts the gel-like, aqueous protein spinning dope within the major ampullate (MA) gland into a super fiber is of great importance for developing future biomaterials based on spider silk. In this work, the initial state of the silk proteins within Black Widow MA glands was probed with solution-state NMR spectroscopy following isotopic enrichment. 15N relaxation parameters, T1, T2 and 15N-{1H} steady-state NOE were measured for twelve backbone environments at two spectrometer frequencies, 500 and 800 MHz. All observed backbone environments are consistent with MA silk protein dynamics on the fast subnanosecond timescale. In addition, the observed conformation-dependent isotropic chemical shifts are consistent with a random coil. Therefore, it is concluded that the repetitive core of spider MA proteins are in an unfolded, highly flexible state in the MA gland. We propose that spider silk proteins can be considered intrinsically disordered proteins prior to fiber formation.

3477-Pos Board B205

Implications of Order Disorder Transitions in the Androgen Receptor for the Onset and Treatment of Late Stage Prostate Cancer

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The nuclear hormone receptor androgen receptor (AR) is a transcription factor of 919 amino acids activated by androgens that regulates the development of the male sexual phenotype [1]. It is composed of three domains: a flexible N-terminal intrinsically disordered transactivation domain that interacts with the transcription machinery, a DNA-binding domain that binds specific DNA sequences adjacent to genes regulated by AR and a C-terminal domain that upon androgen binding undergoes a structural change that activates the protein. To understand the structural basis of transcription activation by the N-terminal domain of AR we have used NMR. We have in particular characterized the interaction between the N-terminal intrinsically disordered transactivation domain and the C-terminal domain of subunit 1 of TFIIF, a general transcription factor tightly associated with RNA polymerase and therefore considered part of the transcription machinery [2].

We find that this protein-protein interaction induces structure in an otherwise disordered region of sequence of this domain and have produced a structural model for the complex that we have validated both in vitro and in vivo. As we will show our results provide information about the mechanism by which AR activates transcription in castration resistant prostate cancer, a late stage of the disease for which there currently is no treatment.

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3478-Pos Board B206

The Intrinsically Disordered Photosystem II Subunit, PsbO, is a Sensor for the Hydrogen Bonding Network in the Oxygen Evolving Complex

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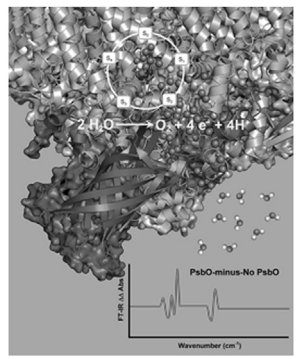
In Photosystem II (PSII), molecular oxygen is evolved from water after four sequential light-driven reactions, producing five oxidized states, Sn. PSII is composed of membrane-spanning subunits and an extrinsic subunit, PsbO. PsbO is intrinsically disordered and plays a role in facilitation of the water oxidizing cycle. Native PsbO can be removed and substituted with recombinant PsbO, thereby restoring steady-state activity. We used reaction-induced Fourier transform infrared (FT-IR) spectroscopy to obtain information concerning the role of PsbO during the S state cycle. Light-minus-dark difference spectra were acquired. A comparison of S_2-minus-S spectra demonstrated that amide frequency and intensity changes were associated with PsbO removal. These data suggest that PsbO acts as an organizational template for the PSII reaction center. To identify any coupled conformational changes arising directly from PsbO, global ^{13}C PsbO isotope-editing was employed. The reaction-induced FT-IR spectra of accessible S states provided evidence that PsbO spectral contributions are temperature (263 and 277 K) and S state dependent. These experiments suggest that PsbO samples a rough conformational landscape when bound to its target, the photosystem II reaction center.



3479-Pos Board B207

The Intrinsically Disordered Cytoplasmic Domain of the T-Cell Receptor Zeta Subunit Does not Form Disordered Dimers

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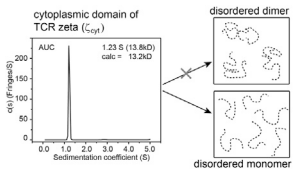
Intrinsically disordered proteins play important roles in recognition, signal transduction and molecular sorting. They are generally thought to interact via coupled folding and binding, yielding largely ordered interfaces with their binding partners. In contrast, the intrinsically disordered cytoplasmic domain of the T-cell receptor zeta subunit (ζ_{cyt}) was reported to form homodimers with a dissociation constant of ~10μM in the absence of a disorder-to-order transition. This suggested the existence of highly disordered dimers. We show here using analytical ultracentrifugation that ζ_{cyt} is a disordered monomer up to at least millimolar concentrations. Paramagnetic relaxation enhancement demonstrates

transient intra- rather than intermolecular interactions. Furthermore, even disulfide crosslinking of ζ_{cyt} N-termini, in a configuration reminiscent of T cell receptor clustering, fails to lead to an association of protomers. SEC-MALS confirms the monomeric state of ζ_{cyt} but reveals a curious concentration-dependent shift of the elution volume of ζ_{cyt} that may previously have been interpreted as dimerization. Our data show that ζ_{cyt} does not form a highly disordered protein complex but leave open the question as to whether completely disordered dimers or other oligomers exist in nature.



3480-Pos Board B208

Mapping Residual Structure in Disordered Protein Ensembles with Millisecond H/D Exchange Mass Spectrometry

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Interactions of intrinsically disordered proteins (IDP) with their binding partners often involve coupled binding and folding. A long-standing question is the extent to which folding of the IDP is mediated by selection of a folded conformer from the disordered state ensemble rather than folding induced by interaction with the binding partner. Answering this question requires detailed information about the disordered state ensemble, in particular the extent to which the IDP possesses residual structure. Yet obtaining this type of information at near-atomic resolution remains challenging. To address this need, we have developed an approach based on millisecond quench-flow amide H/D exchange and mass spectrometry to measure residual structure. In the present work, we examine residual structure in the disordered CBP-binding domain of ACTR as a model system for validation. Following millisecond H/D exchange and acid quench, digestion with pepsin produced a set of 67 highly-overlapping fragments covering the entire 77-residue sequence. Residue-by-residue analysis of empirically-determined H/D exchange half-life obtained from each ACTR fragment provided exchange kinetics at near-residue resolution.

In ACTR, we found that the regions that are known adopt an α-helical fold upon binding to CBP became more protected from H/D exchange than the structured loop regions. We also found that most of the N-terminal region, which does not appear in the solved structure, was the least protected. There was also evidence of slight protection in a short stretch of the N-terminal region. Our results are consistent both with a recent analysis of residual structure obtained from NMR secondary shift measurements and with the AGADIR helicity prediction algorithm. Our results demonstrate the utility of millisecond H/D exchange for mapping secondary structural propensity in disordered state ensembles with near-residue resolution.

3481-Pos Board B209

Structure and Internal Dynamics of Calcitonin Family Peptides: Implications for Amyloid Formation

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The calcitonin peptide (Ct) family comprises the intrinsically disordered proteins amylin (IAPP), calcitonin gene-related peptide (CGRP), calcitonin, and adrenomedullins. These are genetically and structurally related hormone peptides that are able to bind to each other's receptors, though with varying degrees of affinity. Some of these peptides form amyloid fibers, while others do not. They contain highly conserved sequence elements that have been experimentally shown to affect the secondary structural preferences of these peptides. The effect of such conserved elements on tertiary structure has not been experimentally explored to the same extent. Detecting tertiary structural properties of IDPs is considerably more challenging due to fast reconfigurations of the backbone over a wide range of possible conformations. High resolution time-resolved techniques are needed. We use a nanosecond laser spectroscopy technique to measure transient tertiary contact formation. This technique reveals information on the structure and internal dynamics of IDPs. We compare members of the Ct family which differ in hydrophobicity and net charge, and study the effect of proline mutations on contact formation rates. We find that functionally required, conserved sequence elements play an important role in determining the structure, internal dynamics and aggregation properties of these peptides.

3482-Pos Board B210

Enantiospecific Recognition of the Intrinsically Disordered C-Myc Oncoprotein by Small Molecules

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The prevalence of intrinsically disordered proteins (IDPs) in cell signaling and disease makes them attractive targets. Despite the absence of defined tertiary structure, small molecules can bind IDPs at sites determined by a short, linear segment of the protein's primary sequence. The oncoprotein c-Myc, a transcription factor that must undergo coupled folding and binding to its obligate partner Max in order to interact with DNA, is an attractive system for understanding specificity in small molecule binding to IDPs. Three independent small molecule binding sites exist in the bHLHZip region of c-Myc, the segment necessary for coupled folding and binding to Max. The chiral small molecule 10074-A4 recognizes one of these sites. A racemic mixture of 10074-A4 exhibits a circular dichroism signal upon binding to c-Myc indicating an enatioselective interaction of the molecule with the protein. We provide a model for this induced circular dichroism signal based on conformational selection in the binding enantiomer, and we synthesize the pure enantiomers and compare their binding. Derivatives of 10074-A4 were synthesized and these also display enatiospecific binding. Even though c-Myc is disordered, and remains so in the complex, it specifically recognizes both the chemical functionalities in the small molecule and their particular three dimensional arrangement.

3483-Pos Board B211

Characterization of the Intrinsically Disordered Region of the Soluble Guanylate Cyclase Alpha-1 Subunit

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Soluble guanylate cyclase (sGC) is a heterodimeric protein, which is activated by nitric oxide, stimulating the conversion of guanosine triphosphate (GTP) to guanosine monophosphate (cGMP). Decreased sGC activity is linked to atherosclerosis, aging, loss of memory, acute ischemia, while increased sGC catalytic activity is associated with endothelial cell proliferation, vasodilation, cell motility and survival. The activation, catalytic activity and structure of sGC are widely studied, yet the mechanism behind sGC regulation is not well understood. Soluble guanylate cyclase contains four distinct functional domains: the H-NOX, Per Arnt Sim, coiled-coiled and C-terminal catalytic domains. The H-NOX domain of the alpha-1 subunit also contains an intrinsically disordered region (IDR) whose role is not understood since it does not affect the dimerization or catalytic activity of the enzyme. Intrinsically disordered regions are regions that have no defined structure but retain function in a highly flexible state. These regions often undergo conformational changes upon protein-protein interactions. The human sGC IDR is predicted to be the first 69 residues of the alpha-1 subunit's amino terminus but a similar region is not found on the beta-1 subunit. The goal of this study is to characterize the secondary structure of this IDR and probe for protein-protein interaction. The sGC IDR is predicted to contain about 26% alpha helical characteristics utilizing secondary structure prediction algorithms. Circular dichroism studies on the sGC IDR have shown that this region contains a small amount of stable helical structure. It also contains transient helical structure that can be stabilized by 2,2,2-trifluoroethanol. Yeast two-hybrid studies are ongoing to identify interacting partners.

3484-Pos Board B212

The Structural and Kinetic Ensemble of ASB9's N-Terminus and It's Role in Substrate Recognition

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Intrinsically disordered proteins (IDPs) have structural ensembles which lack stable tertiary and secondary structure under physiological conditions. IDPs have remolded the structure-function paradigm, often participating in one-to-many and many-to-one protein interactions. One class of IDPs which have remained largely unstudied are the ankyrin repeat and SOCS (supressor of cytokine signaling) box containing proteins (ASBs). This class of proteins have an intrinsically disordered N-terminus, a six ankyrin repeat domain (ARD), and a C-terminal SOCs box domain. The SOCS box of ASB9 is